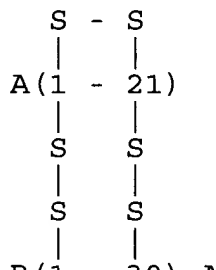
carboxypeptidase B at a pH of about 6.8 [under conditions where no crystals are formed to produce a mono-Arg-insulin; and

(d) cleaving the resulting mono-Arg-insulin with carboxypeptidase B].

27. (Twice Amended) A method as claimed in claim 26, wherein [steps (c) and] step (d) [are] is carried out in one vessel without having to isolate as an intermediate mono-Arg-insulin of the formula II



(II)

REMARKS

Applicants have cancelled claims 29 and 30 and amended claims 21-23 and 25-27. Applicants have amended claims 21, 22, 25, and 26 to include folding and disulfide bridge formation. This amendment is supported at, inter alia, page 3, line 32 to page 4, line 18, and page 15, line 13, to page 14, line 27.

Applicants have amended claims 22 and 26 to recite the trypsin and carboxypeptidase B incubation as one, simultaneous step. This amendment is supported, inter alia, at page 4, line 19, to page 5, line 12.

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Finally, Applicants have amended claims 21, 22, 25, and 26 to delete the lack of crystal formation recital. Applicants added this recital in the last Amendment. This recital was not in the claims as originally filed. Upon entry of this amendment, claims 21-23 and 25-27 will be pending in this application.

Lack of Support Rejection Under 35 U.S.C. § 112, First Paragraph

The Examiner objected to the specification and rejected claims 21-23, 25-27, and 29-30 under 35 U.S.C. § 112, first paragraph for allegedly not being supported by the specification as originally filed. Specifically, the Examiner objected to use of the term "in a native conformation."

Applicants respectfully traverse this ground of rejection. The Examiner contends that mini-proinsulin cannot be "in a native conformation" because it is an insulin precursor not found in nature. Paper 29, page 3, lines 7-11. Applicants respectfully contend that one of ordinary skill in the art would understand that a "native conformation" of a protein not occurring in nature would contemplate a correctly folded protein with disulfide bridges.

Nonetheless, solely to expedite allowance of the pending claims, and not in acquiescence to this ground for rejection, Applicants have amended claims 21, 22, 25 and 26 to delete "in a native conformation," and added folding and disulfide bridge formation. Applicants have also cancelled claims 29-30.

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Therefore, Applicants respectfully request withdrawal of this ground of rejection.

The Examiner also based this section 112, first paragraph, rejection upon the alleged lack of support for the term "under conditions where no crystals are formed." Paper 29, page 2, lines 23-24.

Applicants respectfully traverse this ground for rejection. Nonetheless, solely to expedite allowance of the claims, and not in acquiescence to this ground for rejection, Applicants have amended claims 21, 22, 25 and 26 to delete recitation of "under conditions where no crystals are formed." Therefore, this ground for rejection is moot, and Applicants respectfully request its withdrawal.

Anticipation Rejection of claims 29 and 30 by Grau under 35 U.S.C. § 102(e)

The Examiner rejected claims 29 and 30 under 35 U.S.C. § 102(e) as being anticipated by Grau ('332).

Applicants respectfully traverse this rejection. Applicants have pointed out numerous differences between their compounds and those of Grau.

Nonetheless, solely to expedite allowance of the pending claims, and not in acquiescence to this rejection, Applicants have cancelled claims 29 and 30 from the present application. Applicants reserve the right to present this subject matter in a later-filed divisional or continuation application or in the

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1300 I STREET, N. W.
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present application at a later time. Therefore, this rejection is moot, and Applicants respectfully request its withdrawal.

Alternative Obviousness Rejection of Claims 21-23 and 25-27 under 35 U.S.C. § 103

The Examiner alternatively rejected claims 21-23 and 25-27 under 35 U.S.C. § 103 as being obvious over Markussen et al. (4,916,212) or Markussen et al. (EPO 163,529) either in view of Goeddel et al. (EPO 055,945), Mai et al., Grau (4,801,684), and Grau (4,639,332). The Examiner stated that this rejection would be "essentially as applied to the claims in the prior Office Action." Paper 29, page 4, lines 23-24. The Examiner stated this rejection but did not reject these claims on these grounds because the Examiner considered the previous limitation "native conformation" and the lack of a recited refolding step to render the claims unobvious. In view of the amendments to the claims, Applicants will address this ground for rejection.

Applicants respectfully traverse this rejection. As stated in the previous Amendments, Applicants assert that there are numerous differences between their invention and the applied prior art. Applicants also contend that there would have been no suggestion in the applied prior art to try Applicants' invention nor would there have been any motivation to try their invention.

The previous Office Action, paper 22, rejected the claims over the Markussen references for the reasons in earlier Office Action, paper 19. In paper 19, the Examiner argued that "Markussen et al. ('212) discloses and claims insulin precursors

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& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
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of the form B(1-29)X_n-Y-A(1-21). 'X' is a peptide chain with n amino acids, 'n' is an integer from 0 to 33, and 'Y' is Lys or Arg. X is preferably selected from the group consisting of Ala, Ser, and Thr." Paper 19, page 3, lines 7-11. The Examiner also stated that "Markussen et al. ('529) teaches essentially the same invention." Id. at lines 21-22.

Though Applicants have cancelled their product and product-by-process claims, Applicants continue to maintain that the insulin precursors of both Markussen references do not render their mono-Arg-insulin obvious. Further, since the insulin claims relate to a process of using mono-Arg insulin, the nonobviousness of the mono-Arg insulin product itself is relevant to the nonobviousness of the process of using the mono-Arg insulin. In re Pleudeman, 910 F.2d 823 (Fed. Cir. 1990).

As Applicants have previously argued, it is well established that disclosure of a generic formula does not necessarily render a species of the disclosed genus obvious. In re Jones, 21 U.S.P.Q.2d 1941, 1943 (Fed. Cir. 1992). In Jones, as here, a reference disclosed a broad generic formula encompassing Applicants' claimed species, but only expressly disclosed a few species, not including Applicants'. Id. at 1942. The Federal Circuit reversed the Board's affirmance of the Examiner's rejection, expressly rejecting the PTO's assertion that the size of the previously disclosed genus is irrelevant to obviousness.

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Therefore, Markussen's disclosed generic formula does not necessarily render Applicants' species obvious, and the size of the genus should be considered in determining obviousness.

For Markussen's generic formula to anticipate Applicants' compound, Markussen's "X" would have to be "Thr," one of 3 possibilities; Markussen's "n" would have to be "0," one of 34 possibilities; and Markussen's "Y" would have to be "Arg," one of 2 possibilities.

Applicants have previously pointed out the extremely small odds of one of ordinary skill in the art selecting Applicants' mono-Arg-insulin based on the large ranges and number of variables in Markussen's generic formula. March 25, 1994 Amendment, page 10, line 15 - page 11, line 9. Thus, the nonobviousness of Applicants' mono-Arg insulin compound supports the nonobviousness of Applicants' claims using mono-Arg insulin, claims 22, 23, 26, and 27.

Applicants also assert that one of ordinary skill in the art would have been dissuaded from methods that use a mono-Arg mini-proinsulin as presently claimed.

Those skilled in the art knew that trypsin would not cleave a mini-proinsulin with an Arg-Arg or Lys-Arg bridging C chain. Thim et al., Secretion and processing of insulin precursors in yeast, Proc. Natl. Acad. Sci. USA 83:6766-6770 (1983) at the paragraph bridging pp. 6769, first two sentences (copy enclosed). Similarly, as Applicants have informed the undersigned, it is common knowledge in the art that the basic amino acids Arg (B22)

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and Lys (B29) of the B chain are not readily accessible to trypsin.

Since it was well known that B and A chains linked by Arg-Arg or Lys-Arg were not susceptible to trypsin cleavage, one skilled in the art would not have been given a reasonable expectation that mono-Arg mini-proinsulin used in the presently claimed method would be susceptible to trypsin cleavage, despite the Markussen references' generic formula. In fact, one of ordinary skill in the art would have expected no cleavage by trypsin.

Finally, Applicants' method of making insulin claims, claims 22 and 26, from which claims 23 and 27 depend, have been amended to recite simultaneous trypsin and carboxypeptidase B incubation. This simultaneous incubation is nowhere taught or suggested by the Markussen references, nor do the other references correct this deficiency.

As disclosed in the specification, previous insulin production methods involve a two-step reaction. Page 4, line 19, to page 5, line 12. Specifically, after tryptic cleavage, insulin-Arg^{B31}-Arg^{B32} and mono-Arg insulin are separated from the undesired Des-B30 via ion exchange. The desired insulin-Arg^{B31}-Arg^{B32} and mono-Arg insulin are then cleaved by carboxypeptidase B to yield insulin.

In contrast, Applicants' process of claims 22 and 26 surprisingly yields "hardly any insulin Des-B30." Page 4, lines 36-37. Therefore, Applicants' process does not require the laborious previously-used process that included separating the

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1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

desired products resulting from the trypsin lysis. Rather, Applicants' process obviates the need for this separation and allows the trypsin and carboxypeptidase B reactions to occur simultaneously. Page 5, lines 9-12.

Not only do the cited references fail to teach or suggest such a simultaneous reaction, but they cannot even utilize it. Though Markussen et al. ('332) does not produce insulin, this reference does require cation exchange to purify the products of trypsin cleavage. Col. 4, line 1. Thus, the process of the '332 patent cannot simultaneously involve trypsin and carboxypeptidase B incubation.

The process of the Markussen et al. ('529) cannot involve simultaneous trypsin and carboxypeptidase B incubation to yield insulin because no insulin would be formed. The insulin precursors of this reference are missing Thr^{B30}. This is evident from the stated need for the laborious transpeptidation of the precursor with a L-threonine ester in the presence of trypsin or a trypsin derivative, followed by transformation of the threonine ester to human insulin. Page 8, lines 20-24. Therefore, simultaneous incubation with trypsin and carboxypeptidase B would not yield insulin.

As discussed in the responses to previous Office Actions, the other references cited in this rejection do not remedy the deficiencies of the Markussen et al. references. Therefore, Applicants respectfully request withdrawal of this rejection.

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

Conclusion

In view of the foregoing amendments and remarks, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of the pending claims.

If there are any other fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 06-0916. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER

By: Michael T. Siekman
Michael T. Siekman
Reg. No. 36,276

By: M. Paul Barker
M. Paul Barker
Reg. No. 32,013

Dated: November 6, 1995

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000